#10 7/26/99

PATENT
ATTORNEY DOCKET NO. 07043/015007

Declaration

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : M. Allen Northrup et al. Art Unit: 1634
Serial No.: 08/900,735 Examiner: Sisson, B.
Filed : 07/24/1997
Title : MICROFABRICATED REACTOR

Assistant Commissioner for Patents
Washington, DC 20231

DECLARATION UNDER 37 CFR § 1.131

I, Richard M. White, declare as follows:

1. I am a coinventor of the invention described in the claims of the above-identified patent application, as amended by the Response filed herewith.

2. Prior to May 1, 1992, Dr. M. Allen Northrup ("Dr. Northrup") and I completed the conception of the invention in this country as evidenced by the following:

- Prior to May 1, 1992, Dr. Northrup and I wrote an Invention Disclosure Statement entitled "Microinstrumentation-based Polymerase Chain Reaction (PCR) Diagnostics" (Exhibit A). The Invention Disclosure Statement describes the features of an instrument for amplifying a preselected polynucleotide in a sample. This instrument included a reaction chamber and at least one reactant chamber, at least one channel interconnecting the reaction and reactant chambers, a heater configured to heat reactants in the reaction chamber, a temperature controller coupled to the heater and configured to control the temperature of a reaction in the reaction chamber, and a product analysis chamber coupled to the reaction chamber and adapted to analyze reaction products contained in the product analysis chamber, as recited in independent claims 1 and 104 of the above-identified application.

3. Dr. Northrup and I worked on the invention with due diligence in this country until the invention was reduced to practice prior to May 1, 1992, as evidenced by the following:

- In a notebook I kept, an entry (Exhibit B) dated before May 1, 1992, describes planned operational tests on a PCR instrument ("instrument A") that embodies each of the features recited in independent claims 1 and 104, and was constructed and tested prior to May 1, 1992.
- In a notebook kept by Dr. Northrup, an entry (Exhibit C) dated before May 1, 1992, describes the results of certain operational tests on instrument A. In particular, instrument A was successfully operated to amplify a preselected nucleotide.
- In the above-mentioned notebook kept by Dr. Northrup, an entry (Exhibit D) dated before May 1, 1992, contains a description of another instrument ("instrument B") that embodies each of the features recited in independent claims 1 and 104, and was constructed and tested prior to May 1, 1992.
- In the above-mentioned notebook kept by Dr. Northrup, an entry (Exhibit E) dated before May 1, 1992, describes the results of certain operational tests on instrument B. In particular, instrument B was successfully operated to amplify a preselected nucleotide.

4. Each of the dates deleted from Exhibits A-E is prior to May 1, 1992.

5. I hereby declare that all statements made of our own knowledge are true and that all statements made on information and belief are believed to be true. I understand that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. § 1001) and may jeopardize the validity of the application or any patent issuing thereon.

Date: 12 July 1999 Richard M. White

Richard M. White

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EXHIBITS FOR DECLARATION OF RICHARD M. WHITE

Date of Deposit 7/12/99
I hereby certify under 37 CFR 1.8(a) that this correspondence is being deposited with the United States Postal Service as **first class mail** with sufficient postage on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Sheri Wysocki
SHERI WYSOCKI

A

Invention Disclosure Statement

Title: Microinstrumentation-based Polymerase Chain Reaction (PCR) Diagnostics

Inventors:

M. Allen Northrup
923 Creston Rd
Berkeley, CA 94708

Richard M. White
350 Panoramic Rd
Berkeley, CA 94708

Date:

Background:

The polymerase chain reaction (PCR) is a method by which a single molecule of DNA (or RNA) of an organism can be selectively amplified several millionfold within a few hours. This well-established procedure is based on the repetition of heating (denaturing) and cooling (annealing) cycles in the presence of the original DNA molecule, specific DNA primers, dNTPS, and DNA polymerase enzymes. Each cycle produces a doubling of the target DNA segment, leading to an exponential accumulation of the target segment. The generalized procedure involves: 1) processing of the sample to release target DNA molecules into a crude extract, 2) addition of an aqueous solution containing enzymes, buffers, deoxyribonucleotide triphosphates (dNTPS), and two oligonucleotide primers, 3) thermal cycling of the reaction mixture at two or three temperatures (i.e., 94, 72, and 37-54 °C) for typically 20 to 30 cycles, and 4) amplified DNA detection. Intermediate steps are introduced in some assays to incorporate signal-producing and/or surface-binding primers, and to purify the reaction products (e.g., electrophoresis or chromatography. Reaction volumes and times are typically on the order of tens of μLs and one to two hours, respectively. PCR-based technology has been applied to a variety of analyses, including environmental and industrial contaminant identification, medical diagnostics, and biological research.

Monolithic microfabrication technology has advanced to the point where a variety of of micro-scale components can be made that have electrical, mechanical, optical, chemical, and thermal capabilities. For example, devices have been fabricated that can pump, heat, cool, and mix microliter quantities of solids and liquids. As well, micro-scale optical and electromechanical/chemical physical and chemical sensors have been developed such as fiber optic probes and Lamb-wave sensors. The integration of these devices into systems allows the development of analytical instruments on a micro-scale. The advantages of such integrated devices include the ability to manufacture them in batch quantities with high precision, yet low cost. Their inherent small size also provides significant advantage in that they would be able to perform highly automated *in situ* analyses.

Invention Concept

The invention disclosure herein concerns the application of microinstrumentation to PCR. The small analytical and reaction volumes of PCR make it an ideal diagnostic technique for

implementation on micro-devices. Such a system could contain reservoirs of reagents, agitation and mixing devices to process the target cells, pumps to carry solid and/or fluid reagents to mixing chambers, heaters and coolers to perform the denaturing and annealing cycles, optical and/or electromechanical/chemical sensors to discriminate the reagents and products of the reaction, and separation devices to purify reactants and products. Feedback control via integrated sensors could also be incorporated directly into the system.

Many or all of of these devices could be made from microfabrication technology and could process micro- to picoliter volumes. By the selection and integration of appropriate microfabricated devices, a precise and reliable reaction and analysis instrument for PCR-based diagnostics could be devised. A schematic diagram of an example of one such possible system is presented in Figure 1. Several to many of these micro-instruments could be manufactured on a wafer and could run in parallel, allowing the processing and analysis of several target agents and controls. Target DNA detection methodology could include either an optical, electromechanical, electrochemical, or a combination sensing device. Detection signals could be processed and stored with microelectronic devices.

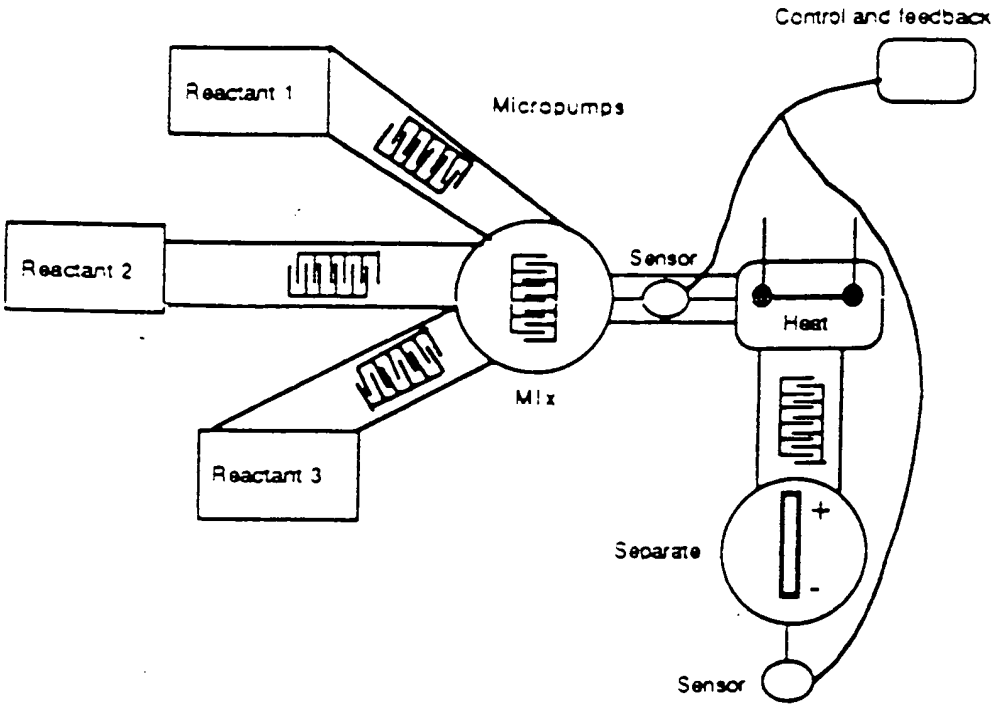


Figure 1. An example of an integrated microinstrument.

In summary, in this disclosure we describe an integrated microsystem and analytical instrument to perform PCR-based diagnostic methodology. The amplification process from minute sample sizes and reaction volumes, and specific reaction sequence of the PCR technique plays favorably into the micro-device capabilities of on-going microfabrication technology. The development of this integrated micro-PCR system will lead to a highly automated, miniaturized, analytical instrument for *in situ* analyses of a variety of samples.

Inventors:

M. Allen Northrup _____ Date _____
923 Creston Rd
Berkeley, CA 94708

Richard M. White _____ Date _____
350 Panoramic Rd
Berkeley, CA 94708

Witnesses:

Christine Saunders _____ Date _____

_____ Date _____

CLASS CODE

UNIVERSITY OF CALIFORNIA, BERKELEY
OFFICE OF TECHNOLOGY LICENSING
INVENTION AND TECHNOLOGY DISCLOSURE

(See instructions on reverse side)

8.
LOG DATE

COMPLETE ITEMS 1-4. USE ADDITIONAL SHEETS AS NECESSARY

1. TITLE OF INVENTION

MICROINSTRUMENTATION - BASED POLYMERASE CHAIN REACTION (PCR) DIAGNOSTICS

2. INVENTOR(S)	TITLE	CAMPUS UNIT OR MAILING ADDRESS
M. ALLEN NORTHRUP	POST DOCTORAL FELLOW (LLNL); VISITING SCHOLAR (UCB)	LLNL; BSAC
RICHARD M. WHITE	PROFESSOR	BSAC; EECS DEPT.

3. CONTRACT OR GRANT NO.(S)	SPONSOR(S)	PRINCIPAL INVESTIGATOR
N/A	—	—

4. EVENTS

	DATE	REFERENCES & COMMENTS
A. Initial idea		TELEPHONE CONVERSATION BETWEEN THE INVENTORS
B. First description of complete invention, oral or written (conception)*		THIS DISCLOSURE (ATTACHED)
C. First successful demonstration, if any (first actual reduction to practice)*	NOT YET	
D. First publication containing full description of invention (establishment of publication bar)*	NOT YET	
E. External oral disclosures	NONE	

5. BRIEF ABSTRACT OF INVENTION - ATTACH DETAILED DESCRIPTION

INVENTION CONCERNS APPLICATION OF MICRO-STRUCTURES TO INSTRUMENT PCR AMPLIFICATION OF DNA, ETC. A SPECIFIC EMBODIMENT EMPLOYING THE LAMB-WAVE ULTRASONIC TECHNOLOGY IS SHOWN.

KEYWORDS (OTL USE ONLY)

6. INVENTION SUBMITTED BY:

MAN _____ Inventor's Signature _____ Date

R.M. White
Co-Inventor's Signature _____ Date

Co-Inventor's Signature _____ Date

Co-Inventor's Signature _____ Date

INVENTION DISCLOSED AND UNDERSTOOD BY:

Witness Signature _____ Date

Scott A. Ieder Jr.
Print Name

Witness to

Company Witness _____ Date

OTL [illegible]

UNIVERSITY OF CALIFORNIA, BERKELEY (UCB)
OFFICE OF TECHNOLOGY LICENSING

AGREEMENT CONCERNING DEVELOPMENT OF TECHNOLOGY AND DISTRIBUTION OF INCOME

Case No. B _____

Name of Technology: Microinstrumentation-Based Polymerase Chain Reaction PCR) Diagnostics

Creators: M. Allen Northrup and Richard M. White

Reference: University of California Patent Policy as revised.

1. UCB and Creator(s) desire that the above Technology be licensed by UCB to industry in order that applications and uses of the Technology be made widely available for public use and benefit. Creator therefore assigns to UCB any right, title, and interest he or she may have in the Technology including, but not limited to, patent, copyright, tangible research materials, and semiconductor mask work rights, and assures UCB that he or she has not granted any such rights in Technology to any other person or entity. The term "tangible research materials" refers to research results which are in tangible form as distinct from intangible (or intellectual) property. Examples include integrated circuit chips, computer software, biological organisms, engineering prototypes, engineering drawings and other property which can be physically distributed.

2. UCB shall take such actions as it believes appropriate to make the Technology available for public use and benefit, but shall not be liable for any failure to generate income thereby.

3. Creator agrees to cooperate with UCB to secure and protect UCB's interest and ownership in the Technology, including executing patent assignment and other pertinent documents, giving testimony, and providing pertinent information; provided, however, that if any expenses are incurred by Creators in providing such cooperation, such expenses shall be paid by UCB.

4. Considering the foregoing, Net Royalty Income will be distributed as follows:

CREATOR(S) SHARE: 33 1/3% of Net Royalties
DEPARTMENT SHARE: 50% of Adjusted Net Royalties
UNIVERSITY SHARE: 50% of Adjusted Net Royalties

The academic department(s) (or organized research unit(s)) of the creators are:

Electrical Engineering and Computer Sciences Department and

Berkeley Sensor and Actuator Center

5. "Net royalties" are defined as gross royalties and fees, less 15% thereof for administrative costs, and less the out-of-pocket costs of patenting, protecting, and preserving patent rights, maintaining patents, the licensing of patent and related property rights, and such other costs, taxes, or reimbursements as may be necessary or required by law, and a reserve to cover out-of-pocket expenses which UCB reasonably determines may be incurred in following fiscal years which may not be covered by future royalty revenue. When no longer needed, UCB agrees to distribute the balance of funds reserved according to the formula of paragraph 4 above.

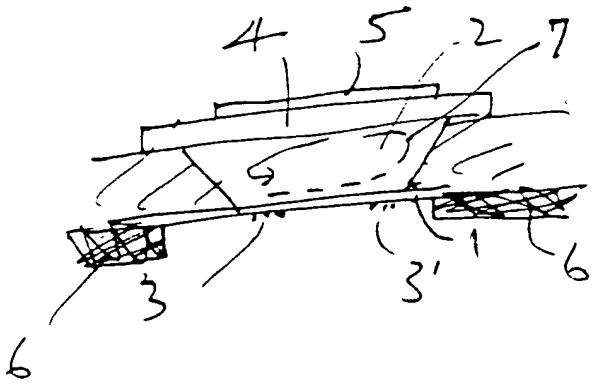
6. "Adjusted Net Royalties" are defined as "Net Royalties," as specified in Paragraph 5 above, less the following deductions to such Net Royalties thereby calculated:

Revlite

Q: any space tests available? Phoned Rudy Paulhalzer yesterday to inquire.

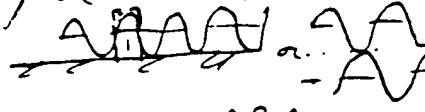
A.N./BMS:

Could do LW PCR test with existing device by adding on top a foil heater – either as is (try PCR regular tubes first) or attach to a cover slip!



1 membr.
2 reactants
3, 3' xducers
4 cover
5 heater (could combine 4 and 5.
6 heater for the lower (or higher) temp.
7 circulation pattern

← To change drive via a phase shift = to one xducer also possible. Changing freq of one xducer slightly would give a control knob to trim or pump velocity as it varied depth of "pumping" region. (←as on 2pp back)



Pump flow rate: If channel measures 3 mm × 2000 Å & v_{wave} = 400 m/s, flow rate is

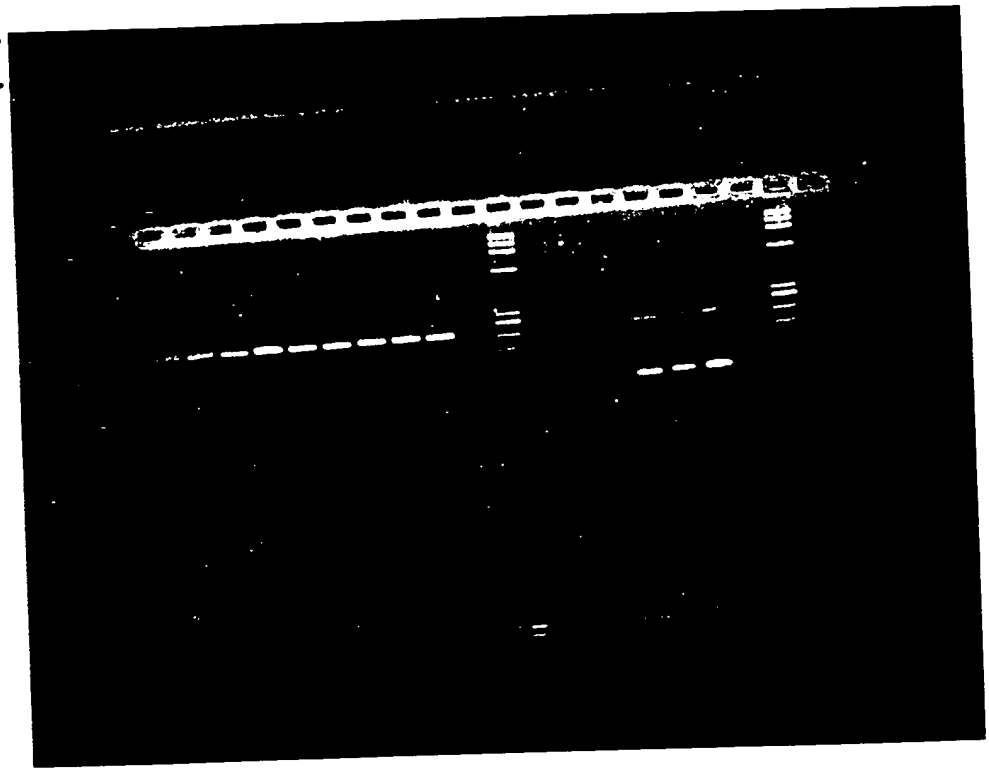
$3\times10^{-1}\,cm \times 2\times10^{-5}\,cm \times 4\times10^{4}\,cm/s = 24\times10^{-2}\,cm^3/s$

$= 0.24\,cm^3/s \Rightarrow 240\,\mu L/s.$

C

M. Allen

Device wells



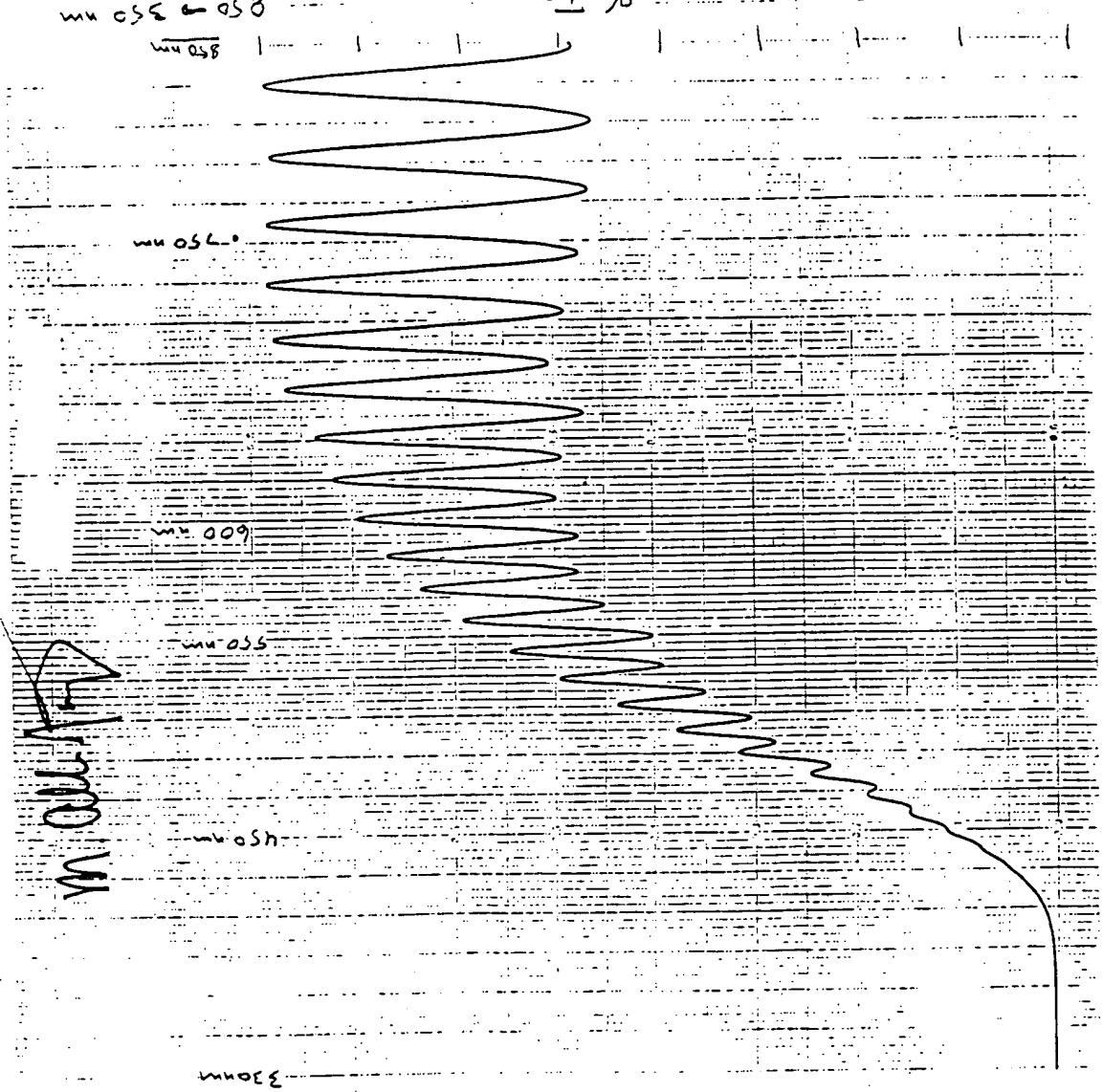
Duplicate PCR - Device M. Allen

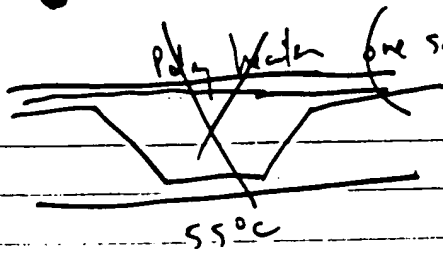
Notes (rewritten M. Allen)

Device PCR:

1) PCR worked in 20-25 µl rxn volume w/ > 200 µl oil

* 2) amplification occurred on both target and on primers (Primer-Dimers) probably due to ΔT in device: i.e., did not reach 96°C even though cycler was set to 99°C.

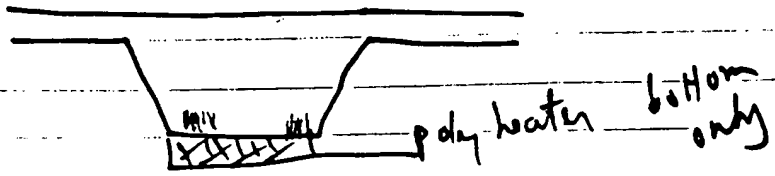




M. Allen

I-V → temperature used to calibrate

resistance vs ~~temp~~ Temp.



Next
- Foil heater on top (effect of foil heater on reaction?)
- Pump
- ~~Entrance~~ Foil Top heater
  - Find 4°c refrigerator (Microlab?)

To do soon:
1) different - Surface/volume
2) _____ - device w/ heater on bottom
   - Standard device first

ask Otus
- borrow cycler ~ 2 weeks

E

@ Cetus M. Alt

Try new PCR system
(more Temp. forgiving)

142 bp product target on SS M13 from gag- region of HIV

1) Starting target = 10^8 copies in 5 µl
T = 96-55 (works at 88+) → 16-18 cycles is plenty

2) primers

old names:		new names	
SK 145	=	ph 07	10 pM/µl
SK 431	=	ph 08	

Reaction mixture: (500 µl)

50 µl	10 x Buffer w/ mgcl	
"	1 mM dntps	
"	M13 w/ gag region of HIV	
10 µl	= 10 * 10 =	100 pmoles
	ph 07	
10µl (Same For)	ph 08	?
2½ µl	= 10 x 1.25 µ/pml	12.5
	Taq	
327.5	ddH_2O	
500 µl	total rxn volume	

0
50
172½
327.5

M. allf

1) re-use voltage (same device) as on March 30 (ie 3.17 V ± 98°C) at 0.2A

Do only 20 cycles

A) Standards

10, 10, 20, 20, 30, 30, 40, 40

~150 µl oil (1-8)

B) Device 30 µl w ~ 90 µl oil

1-minute cycles at 3.17 V
20-1 minute cycles (A-E) 0.2A

Electrophresis

well-problem

P std ϕ 1 2 3 4 5 6 7 8 ϕ ϕ A B C D D E + 4 1 ϕ ϕ

1a) Had to re-solder device wire connectors after 2-cycles fix time ≈ ½ hour rxn was at room temp

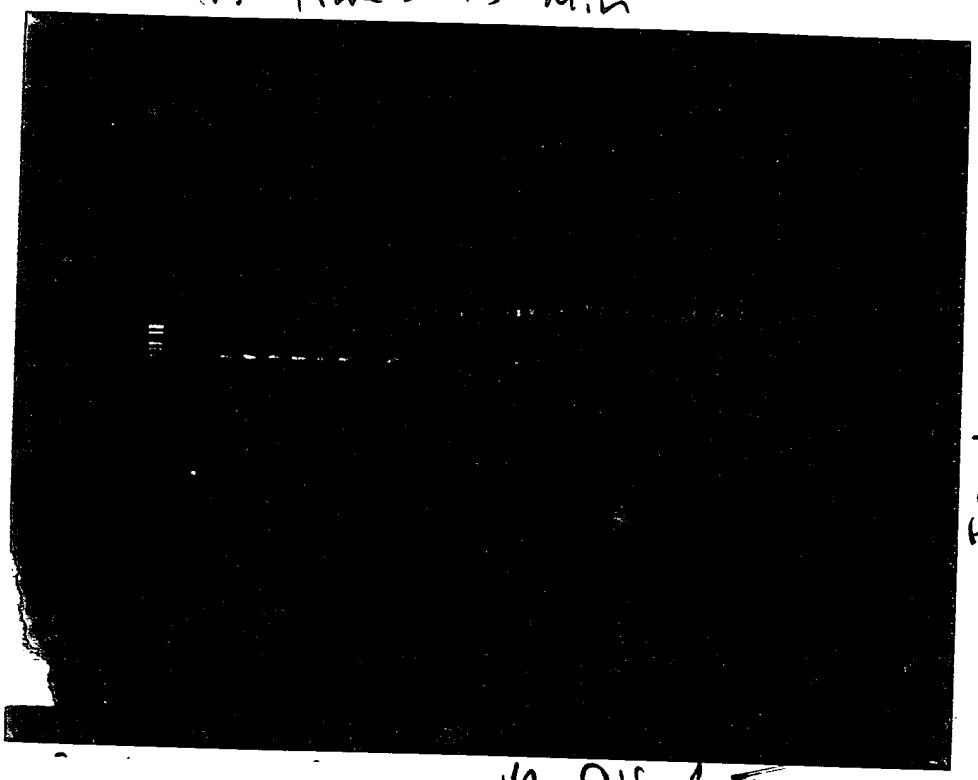
BEST AVAILABLE COPY

Results - ① formed product in both stds and in wells
② well 8 (and 1) std had less bright pr or -Dimers
③ device provided ~6-5 µl gel loadings (one was disrupted)

See next 2 pages:

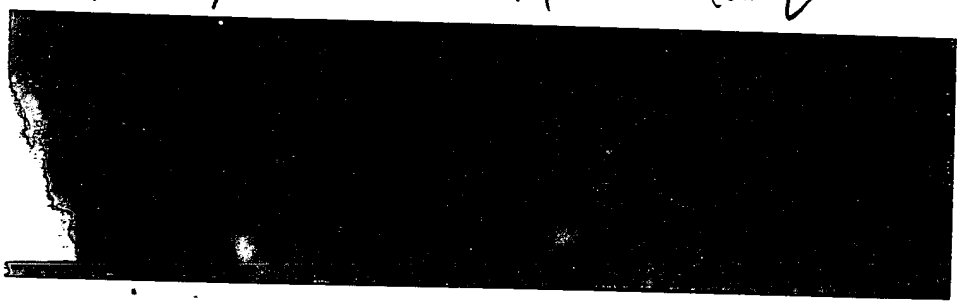
Cont results (photos) M. Allen

Electr. Time = 15 min



T = 1 sec 5.6 3200

M. Allen



T = 1 sec 5.6 3200

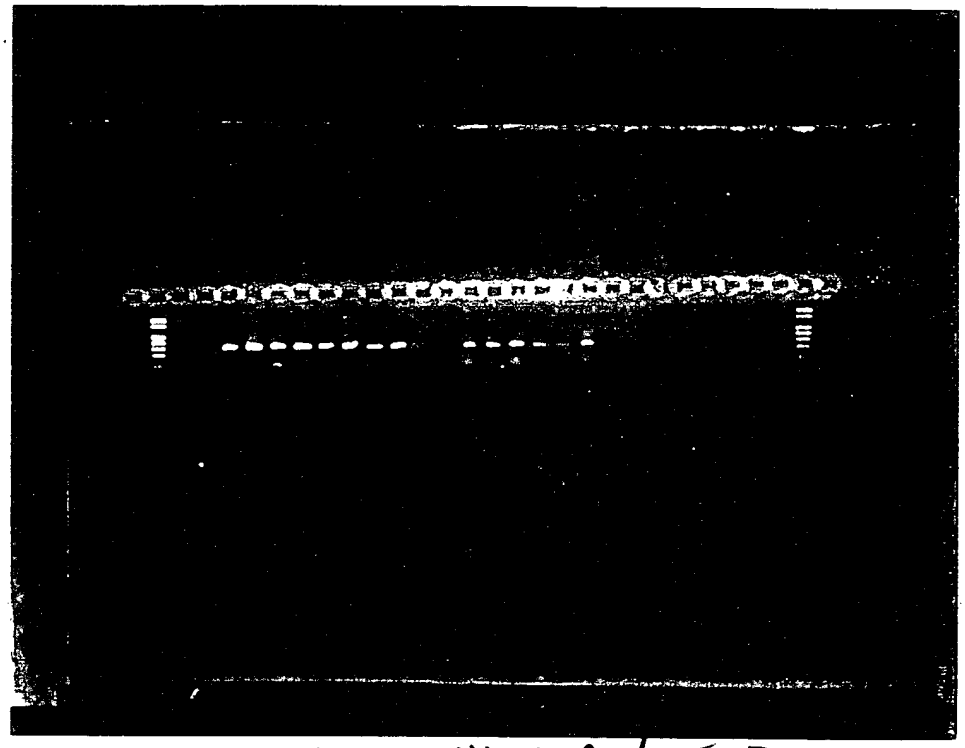
#1 turned in Mid Chem

Cont

Results (photos)
Devia PCE results positive

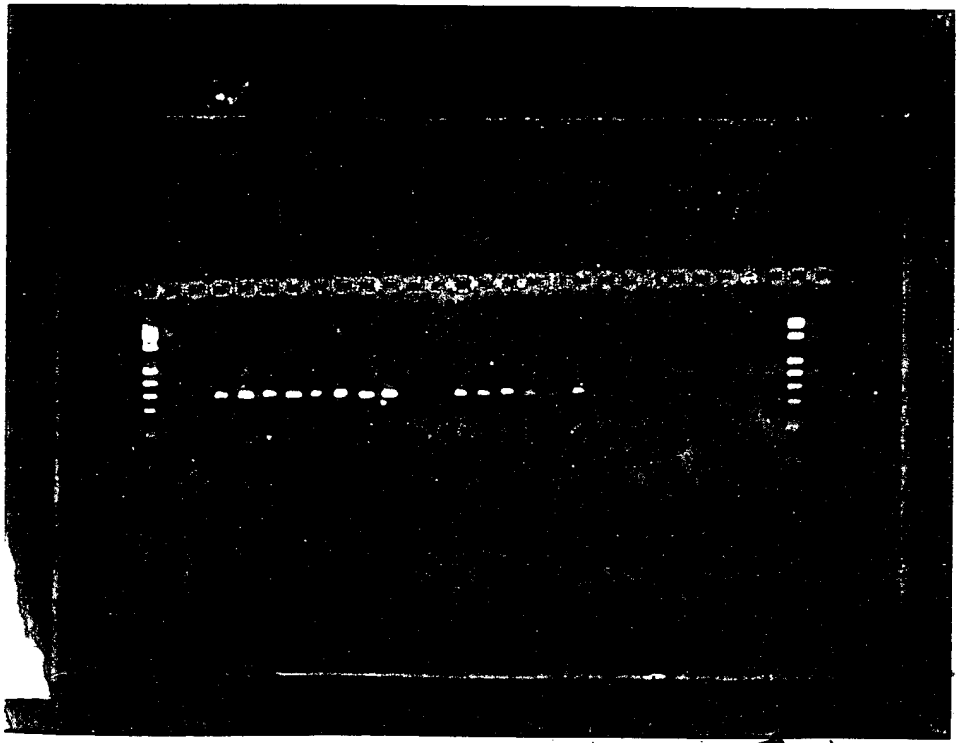
M. Allen

elec. T = 15 min



M. Allen T = 2 sec 4.6 3200

elec T = 40 min



M. Allen T = 1/2 sec 3200

Cont
Results

M. Allen/Mary

Notes (signal book of several (not all) results photos with this pen (often (on front) was ~~was~~ permanent ink)

- PCR (HIV - MSP) worked well in integrated-heater devices, gel electrophoresis verified product. Some, but minimal Primer Dimer (esp. due to known fact that device react. in mixture cycled 1-2 times, then at R.T. for ½ hr & prior to 20 cycles due to need to re-solder connections - new rxn mixture (30 µl) was added)

- was able to extract ~ 100% of aqueous phase with 200 µl (set at 30 µl) pipette & load 5-6 wells of electrophoresis channel

(*) → calculate power consumed in todays experiment compare to betters

Other Discussion

last Tues w/ Ray Marella Lee (Calus) along w/ Russ Higuchi, Bob Watson, Russ's technician, myself we tried homogeneous detection w/ video CCD over 460 thermal cycles

- pulsed N_2-laser (ILEE laser company, Switz) was tried

→ see ~~LLNL Book~~ LLNL (notebook) for details